SYNAPSE GENOMICS CORE FACILITY

DNA Sequencing Run Sheet

Run SGC-RUN-2026-0447 • Vertex ctDNA Biomarker Panel, Cohort 3

Run Identification

Run ID	SGC-RUN-2026-0447
Client / Project	Vertex Therapeutics — ctDNA Biomarker Panel, Cohort 3 (Protocol VX-ONC-114)
Requesting PI	Dr. Owen Baptiste, Translational Oncology Group, Vertex Therapeutics
Sequencing Operator	Daniel Osei
QC Reviewer	Dr. Naomi Frost, Senior Genomics Scientist
Run Start	22 July 2026, 18:42
Run Complete	24 July 2026, 09:15
Instrument	Synapse SG8000 Sequencing System (Unit SG8000-03)
Flow Cell Type	High-Output, 2-lane (SG8000-HO2)
Flow Cell ID / Lot	FC-A00447-000C1 / Lot SG-FC-2607
Reagent Kit Lot	RK-4471-B2 (SBS Chemistry v4.2, 300-cycle)

1 Run Overview

This run sheet documents sequencing run **SGC-RUN-2026-0447**, performed on behalf of the Vertex Therapeutics Translational Oncology Group as part of the ctDNA Biomarker Panel study (Protocol VX-ONC-114, Cohort 3). The objective of this run is targeted amplicon sequencing of a custom 68-gene circulating tumor DNA (ctDNA) panel across eight plasma-derived cell-free DNA libraries, prepared to support variant-calling validation ahead of the Cohort 3 interim analysis.

Libraries were prepared by the Vertex sample-processing team using a hybridization-capture workflow and unique dual-indexed adapters, then delivered to Synapse Genomics Core as normalized, pooled libraries at 4 nM. The core facility performed library QC, pool verification, denaturation, dilution, and loading, and executed the sequencing run described in this document. Full library preparation records are retained separately under Vertex document control (VX-LP-2026-0447) and are referenced here only where relevant to run configuration.

1.1 Sequencing Goal

The panel targets a minimum on-target unique read depth of 8,000× per sample to support variant allele frequencies down to 0.1% VAF, consistent with the sensitivity requirements defined in the study's bioinformatics analysis plan. Read configuration, cluster density targets, and quality thresholds in this run sheet were selected to meet that depth requirement across all eight pooled samples with adequate margin for index-hopping and low-quality read exclusion [2, 1].

1.2 Chain of Custody

- Pooled library received from Vertex courier at 14:10, 22 July 2026 (temperature log attached to sample manifest VX-LP-2026-0447-M).
- Library QC (Qubit fluorometric quantification, fragment analyzer sizing) performed by Daniel Osei prior to denaturation; results recorded in Section 3.
- Post-run FASTQ delivery to Vertex secure transfer endpoint scheduled within 24 hours of run completion per the standing data-transfer agreement.

2 Sequencing Parameters

The following configuration was programmed into the Synapse SG8000 control software prior to run start. All values were verified by the operator against the run worksheet before clicking *Start Sequencing*.

Read Configuration
2 × 151 bp
+ 8 + 8 index reads

Chemistry
SBS v4.2
300-cycle kit

Cluster Density
650–750
K/mm^2 target range

Est. Yield
360 Gb
per flow cell

2.1 Instrument Setup

Parameter	Value
Run Mode	Standard, paired-end, dual-index
Clustering Method	On-board clustering (ExAmp-equivalent bridge amplification)
Base-Calling Pipeline	SeqCall v5.3 (real-time, on-instrument)
Denaturation Protocol	0.2 N NaOH, 5 min at room temperature, 1:1 quench
Loading Concentration	650 pM (post-denaturation, post-dilution)
PhiX Spike-In	1% (v/v), Control Lot PX-2026-118
Custom Primers	None (standard adapter-complementary primers)
Adaptive Loading	Disabled — fixed-volume load per SOP SGC-SOP-014
Secondary Analysis	Demultiplexing + FASTQ generation via SeqCall Local Run Manager

2.2 Denature & Dilution Worksheet

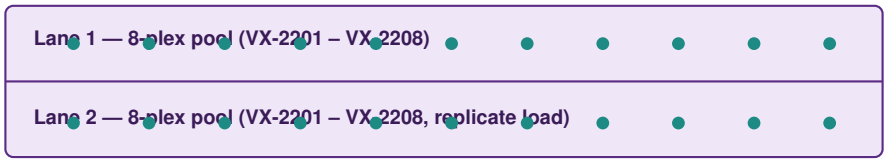
Step	Volume / Conc.	Notes
Pooled library received	4 nM, 20 µL	From Vertex sample-processing team
0.2 N NaOH denaturation	5 µL	5 min, room temperature
Pre-dilution in Tris-HCl	995 µL, pH 8.5	Final denatured pool ≈ 20 pM
PhiX spike-in addition	1% v/v	From 12.5 pM PhiX stock, Lot PX-2026-118
Final dilution to loading conc.	650 pM	Chilled tube, loaded within 30 min of prep

Operator Confirmation

- ☑ Reagent cartridge thawed and inverted 10× per manufacturer instructions.
- ☑ Flow cell inspected for scratches, air bubbles, and lane blockages under inspection lamp — none observed.
- ☑ Run parameters cross-checked against the sample manifest (Section 3) by a second operator prior to run start.

3 Sample Sheet & Indexing

Eight uniquely dual-indexed libraries were pooled in equimolar ratio and loaded across both lanes of the flow cell. Index sequences below were verified against the Vertex library manifest (VX-LP-2026-0447-M) by a second operator prior to denaturation, per SOP SGC-SOP-014 §4.2.



Flow Cell FC-A00447-000C1 • SG8000-HO2

VX-2201
Well **A1** Project **Cohort3-Baseline**
Index ID: **D701–D501**
i7: ATCACGTG
i5: CTAGCTAG
Library conc. **4.1 nM** Input vol. **5.0 µL**

VX-2202
Well **A2** Project **Cohort3-Baseline**
Index ID: **D702–D502**
i7: CGATGTAC
i5: GATCTGAC
Library conc. **3.9 nM** Input vol. **5.0 µL**

VX-2203
Well **A3** Project **Cohort3-Baseline**
Index ID: **D703–D503**
i7: TTAGGCAT
i5: TAGCTAGC
Library conc. **4.3 nM** Input vol. **5.0 µL**

VX-2204
Well **A4** Project **Cohort3-Week4**
Index ID: **D704–D504**
i7: TGACCACT
i5: ACGTACGT
Library conc. **4.0 nM** Input vol. **5.0 µL**

VX-2205
Well **A5** Project **Cohort3-Week4**
Index ID: **D705–D505**
i7: ACAGTGGA
i5: GCTAGCTA
Library conc. **4.2 nM** Input vol. **5.0 µL**

VX-2206
Well **A6** Project **Cohort3-Week4**
Index ID: **D706–D506**
i7: GCCAATGC
i5: CATGACTG
Library conc. **3.8 nM** Input vol. **5.0 µL**

VX-2207
Well **A7** Project **Cohort3-Week8**
Index ID: **D707–D507**
i7: CAGATCTG
i5: TGCATGCA
Library conc. **4.1 nM** Input vol. **5.0 µL**

VX-2208
Well **A8** Project **Cohort3-Week8**
Index ID: **D708–D508**
i7: ACTTGATG
i5: AGTCAGTC
Library conc. **4.4 nM** Input vol. **5.0 µL**

3.1 Index Colour Balance Check

Low base diversity at a given cycle (a single colour channel dominating the flow cell image) degrades base-calling accuracy. Per-cycle nucleotide composition of the Index 1 (i7) read was checked against the facility's minimum-diversity rule (each base ≥5% of reads at every cycle) before the pool was approved for loading.

Cycle	1	2	3	4	5	6	7	8
%A	37.5	12.5	50.0	37.5	12.5	25.0	25.0	12.5
%C	25.0	37.5	25.0	12.5	25.0	25.0	12.5	25.0
%G	12.5	25.0	12.5	25.0	37.5	25.0	25.0	37.5
%T	25.0	25.0	12.5	25.0	25.0	25.0	37.5	25.0

Minimum observed representation: **12.5%** (threshold $\geq$5%) across all eight cycles. Status: PASS

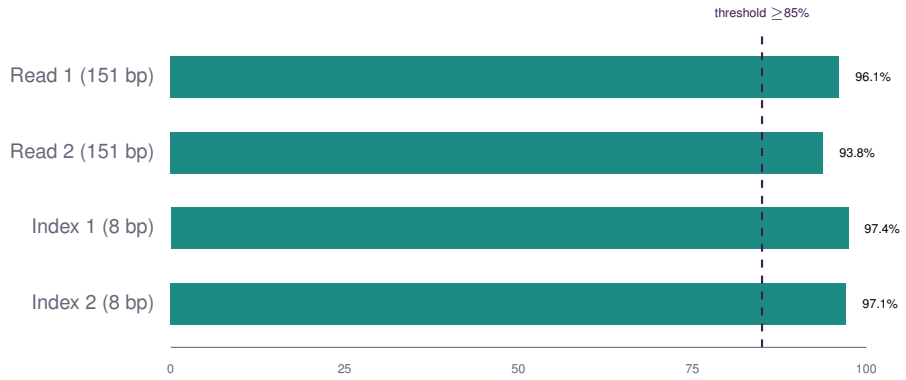
4 Quality Control Metrics

Run-level QC metrics were pulled from the SeqCall v5.3 run report following completion of Read 2 and cross-checked by the QC reviewer against the acceptance thresholds defined in SOP SGC-SOP-021 (*Sequencing Run Acceptance Criteria*). A run is released for demultiplexing and delivery only when every metric below meets or exceeds its threshold.

4.1 Run-Level Metrics

Metric	Threshold	Observed	Status
Clusters Passing Filter (% PF)	≥90%	94.2%	PASS
Cluster Density	650–750 K/mm^2	712 K/mm^2	PASS
Error Rate (PhiX alignment)	≤1.00%	0.42%	PASS
Phasing	≤0.20%	0.11%	PASS
Prephasing	≤0.20%	0.08%	PASS
Undetermined Index Reads	≤5.0%	2.3%	PASS
Total Yield (both lanes)	≥340 Gb	378 Gb	PASS

4.2 Per-Read %Q30



All four reads exceed the 85% %Q30 acceptance threshold with comfortable margin; Read 2 shows the expected modest decline relative to Read 1, consistent with normal sequencing-by- synthesis chemistry decay over longer reads.

4.3 Per-Sample Demultiplexing Summary

Sample	Project	% of Pool	PF Reads (M)
VX-2201	Cohort3-Baseline	12.9%	24.8
VX-2202	Cohort3-Baseline	12.4%	23.9
VX-2203	Cohort3-Baseline	13.1%	25.2
VX-2204	Cohort3-Week4	12.6%	24.3
VX-2205	Cohort3-Week4	12.8%	24.6
VX-2206	Cohort3-Week4	12.2%	23.5
VX-2207	Cohort3-Week8	11.9%	22.9
VX-2208	Cohort3-Week8	12.1%	23.3
	Undetermined	2.3%	4.4

Read 2 %Q30 (93.8%) trended 1.1 percentage points below the prior three runs on this instrument (typically 94.5–95.5%). Value remains well above the 85% acceptance threshold and per-sample yields are within normal variance, so no corrective action was taken. Flagged here for trend monitoring per SOP SGC-SOP-021 §6.3; see also the note in Section 5.

5 Deviations, Review & Sign-Off

5.1 Deviations & Run Notes

- No formal deviations were recorded against SOP SGC-SOP-014 (Sequencing Run Execution) or SOP SGC-SOP-021 (Run Acceptance Criteria) during this run.
- The Read 2 %Q30 trend noted in Section 4 was logged as an observation in the instrument maintenance record for Unit SG8000-03; the reviewer recommends a flow-cell-side inspection at the next scheduled preventive maintenance (due 15 August 2026) rather than an out-of-cycle service call.
- Ambient lab temperature during loading was 21.4 °C, within the 18–24 °C operating range specified for the SBS v4.2 chemistry.

5.2 Disposition

Release Decision

All run-level and per-read QC metrics met acceptance thresholds (Section 4). FASTQ files for all eight samples are **approved for release** to the Vertex Therapeutics secure transfer endpoint and for downstream variant-calling analysis under Protocol VX-ONC-114.

5.3 Review & Signatures

Role	Name	Signature	Date
Sequencing Operator	Daniel Osei	_____	24 Jul 2026
QC Reviewer	Dr. Naomi Frost, Senior Genomics Scientist	_____	24 Jul 2026
Core Facility Director	Dr. Priya Chandrasekaran	_____	25 Jul 2026
Client Acknowledgement	Dr. Owen Baptiste, Vertex Therapeutics	_____	_____

5.4 Distribution

- Original: Synapse Genomics Core Facility QMS archive (retained 10 years per SGC-SOP-002)
- Copy: Vertex Therapeutics Translational Oncology Group, Protocol VX-ONC-114 study file
- Copy: Instrument maintenance record, Unit SG8000-03

END OF RUN SHEET Run SGC-RUN-2026-0447 Synapse Genomics Core Facility

References

[1] Philip Ewels, Måns Magnusson, Sverker Lundin, and Max Käller. MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, 32(19):3047–3048, 2016.

[2] Rounak Sinha, Geoff Stanley, Gunsagar S. Gulati, Camille Ezran, Kyle J. Travaglini, Eric Wei, Charles K. F. Chan, Ahmad N. Nabhan, Tim Su, Rachel M. Morganti, Shannon D. Conley, Hana Chaib, Kristy Red-Horse, Michael T. Longaker, Michael P. Snyder, Mark A. Krasnow, and Irving L. Weissman. Index switching causes "spreading-of-signal" among multiplexed samples in illumina hiseq 4000 dna sequencing. *bioRxiv*, 2017.